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Mx1-Based Resistance to Thogoto Virus in A2G Mice Is Bypassed in Tick-Mediated Virus Delivery

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The interferon-induced mouse Mx1 protein has intrinsic antiviral activity against orthomyxoviruses, including Thogoto virus. Thus, *Mx1*$^+$ A2G mice are apparently resistant to infection following needle- or tick-borne virus challenge. However, tick-borne challenge and, to a lesser degree, injection of virus mixed with tick salivary gland extract resulted in virus transmission to uninfected ticks feeding on the A2G mice. The data indicate that immunomodulatory components in tick saliva can overcome a natural antiviral mechanism.

Resistance of A2G mice to influenza viruses, discovered by Lindenmann (10), is determined by a single gene, *Mx1*, which is structurally altered in influenza virus-susceptible mice. *Mx1* gene expression is induced upon viral infection through the action of type I interferon and encodes a nuclear GTPase, the Mx1 protein (14). Little is known about the mode of action of the Mx1 protein in antiviral activity, except that it interferes with influenza virus primary transcription (8). Recently, it was shown that Mx1 also inhibits the tick-borne Thogoto virus (THOV) (4), which has been classified in a new genus of the *Orthomyxoviridae* family (13).

Nonviremic transmission is defined as transmission of arboviruses between infected and uninfected ticks cofeeding on a vertebrate host that has no detectable, or very low levels of, viremia (12). Nonviremic transmission of THOV between *Rhipicephalus appendiculatus* ticks, the principal vector species of THOV, was first reported to occur on guinea pigs (5). The aim of this investigation was to determine whether nonviremic transmission of THOV occurs on *Mx1*$^+$ A2G mice. This model has advantages over the guinea pig model used previously. First, A2G mice are fully resistant to THOV delivered by either intraperitoneal (3×10^5 PFU), intracerebral (10^3 PFU), or subcutaneous (s.c.) (3×10^3 PFU) injection, whereas similarly challenged *Mx1*$^-$ BALB/c mice develop high levels of viremia and succumb to an acute degenerative hepatitis characteristic of THOV infection (4). Moreover, A2G mice do not sustain detectable levels of virus replication. By contrast, guinea pigs support low levels of THOV replication sufficient to account for low levels of virus transmission to feeding ticks (5). Second, the genetic basis of the resistance to THOV in A2G mice is well described (4), while nothing is known about the mechanisms underlying resistance in guinea pigs. However, a disadvantage of using mice is that the feeding success of *R. appendiculatus* maintained as a laboratory colony is reduced compared to that of guinea pigs and, consequently, a greater number of animals is required for experimentation. All of the ticks were retained within one chamber to maximize the recovery of fed ticks.

Virus injection. In initial experiments, we tested whether THOV injection of A2G mice, compared to similarly challenged BALB/c mice, results in virus transmission to feeding, uninfected *R. appendiculatus* larvae. Stock virus (Thogoto/SiAr/126/72) was prepared in BHK-21 (baby hamster kidney) cells and stored at −70°C at 10^7 to 10^8 PFU/ml as determined by plaque assay in African green monkey kidney (Vero) cells. Female mice (5 to 7 weeks old) were injected s.c. with 5×10^3 PFU of THOV and then immediately exposed to approximately 100 larvae. The ticks were retained on the mice inside neoprene chambers secured on the shaved backs of the animals by using nonirritant latex glue. After 3 days, the larvae had fully engorged and detached, after which the ticks were collected and the mice were killed humanely. The fed larvae were kept for 10 days to allow the acquired virus to multiply and then homogenized in pools of 10 and assessed for virus by plaque assay in Vero cells. Infection levels of the mice were determined by assessing virus content in systemic blood and in the target organs of THOV, the liver and spleen (4). Virus was not detected in blood samples of the A2G mice, and in BALB/c mice, viremic titers were generally 3 orders of magnitude lower than in liver samples (data not shown). All seven BALB/c mice developed infections with virus titers of up to 3×10^5 PFU/g liver, and all of the animals supported virus transmission to the feeding ticks (Tables 1 and 2). In sharp contrast, none of 20 A2G mice showed evidence of infection or allowed virus transmission to ticks (Tables 1 and 2). These results demonstrate that A2G mice are resistant to THOV infection, which is consistent with a previous report (4). The data further show that nonviremic transmission does not occur on A2G mice in response to s.c. needle-borne THOV challenge.

Cofeeding-tick-borne virus transmission. The ability of mice to support nonviremic transmission was tested by cofeeding infected nymphs (donors) and uninfected larvae (recipients) on the same animal. To obtain infected *R. appendiculatus* nymphs, larvae were infected per os by feeding on viremic hamsters and then allowed to molt to nymphs as described previously (1). Ten nymphs and approximately 100 larvae were added simultaneously to the same retainment chamber and allowed to feed until engorgement of the larvae (3 days). All of the BALB/c mice but one became infected, and all seven animals supported virus transmission to larvae (Tables 1 and 2). None of 15 A2G mice developed a disseminated infection, as assessed by titration of blood, liver, and spleen samples. However, following the tick-borne challenge, 5 of the 15 animals supported transmission to recipient larvae (Tables 1 and 2). Virus titers in A2G-derived ticks were comparable to those in ticks fed on BALB/c mice (Table 1). The contrast between the results of the needle-borne and tick-borne challenges did not

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TABLE 1. Transmission of THOV to *R. appendiculatus* larvae

Mode of virus delivery and mouse	No. of donor nymphs recovered	No. of larva pools infected/total no. of pools	Log_{10} PFU/pool of larvae	Log_{10} PFU/g of:	
				Liver	Spleen
5×10^3 PFU injected s.c.					
BALB/c 99	NA[a]	4/4	2.5–3.7[b]	4.6	<1.7
BALB/c 100	NA	4/4	2.8–3.3	4.9	<1.7
BALB/c 101	NA	6/6	2.5–4.5	5.0	3.3
BALB/c 102	NA	3/3	2.7–3.8	5.3	2.6
BALB/c 103	NA	3/3	3.5–3.6	3.0	2.3
BALB/c 104	NA	2/6	2.0–2.9	3.0	2.6
BALB/c 109	NA	2/2	2.3–3.0	4.0	2.3
A2G 1–20	NA	0/90	<0.7	<1.7	<1.7
Cofeeding of infected nymphs					
BALB/c 66	5	1/2	4.0	4.6	3.5
BALB/c 71	3	3/3	2.3–3.5	4.3	3.7
BALB/c 74	1	3/3	3.0–3.5	4.7	4.0
BALB/c 91	6	1/4	4.0	3.3	4.8
BALB/c 93	3	1/4	3.5	<1.7	<1.7
BALB/c 94	5	3/3	5.0–5.5	5.5	5.7
BALB/c 95	6	4/4	3.7–4.3	4.8	4.5
A2G 21	2	0/1	<0.7	<1.7	<1.7
A2G 23	3	2/3	2.1–3.6	<1.7	<1.7
A2G 24	5	3/6	3.3–4.0	<1.7	<1.7
A2G 30	1	0/2	<0.7	<1.7	<1.7
A2G 31	5	0/4	<0.7	<1.7	<1.7
A2G 33	3	0/1	<0.7	<1.7	<1.7
A2G 34	4	3/3	2.9–4.0	<1.7	<1.7
A2G 45	7	1/4	4.0	<1.7	<1.7
A2G 46	6	0/6	<0.7	<1.7	<1.7
A2G 47	5	3/4	2.3–3.8	<1.7	<1.7
A2G 49	5	0/4	<0.7	<1.7	<1.7
A2G 51	2	0/1	<0.7	<1.7	<1.7
A2G 52	4	0/1	<0.7	<1.7	<1.7
A2G 53	5	0/3	<0.7	<1.7	<1.7
A2G 54	6	0/1	<0.7	<1.7	<1.7
5×10^3 PFU + 50 μg of SGE injected s.c.					
BALB/c 96	NA	6/6	3.5–3.9	5.3	<1.7
BALB/c 97	NA	2/2	3.3	4.8	<1.7
BALB/c 98	NA	2/2	3.0–3.5	5.0	<1.7
A2G 84	NA	0/3	<0.7	<1.7	<1.7
A2G 85	NA	0/4	<0.7	<1.7	<1.7
A2G 86	NA	0/4	<0.7	<1.7	<1.7
A2G 87	NA	0/5	<0.7	<1.7	<1.7
A2G 88	NA	0/3	<0.7	<1.7	<1.7
A2G 89	NA	0/2	<0.7	<1.7	<1.7
A2G 90	NA	0/5	<0.7	<1.7	<1.7
A2G 91	NA	2/5	3.3	<1.7	<1.7
A2G 92	NA	2/5	3.3–4.0	<1.7	<1.7
A2G 93	NA	1/4	3.3	<1.7	<1.7

[a] NA, not applicable.
[b] Range of virus titers of infected ticks.

appear to be related to the virus dose. Extrapolation of data on the amount of THOV secreted in the saliva of *Amblyomma variegatum* ticks (7) indicates that an average of approximately 100 PFU was delivered per mouse by the (partially) fed *R. appendiculatus* donor nymphs. Nonetheless, virus transmission was supported by one-third of the animals (Tables 1 and 2). These results demonstrate that nonviremic transmission of THOV can occur with *Mx1*$^+$ A2G mice, although its efficiency is underestimated in this experiment because of the low recovery of engorged larvae and the poor feeding success of the donor nymphs with as few as a single nymph feeding on some animals (Table 1).

Saliva-activated transmission. The underlying mechanism of nonviremic transmission is believed to be a phenomenon called saliva-activated transmission (SAT), i.e., virus transmission potentiated by immunomodulatory components present in tick saliva that facilitate blood feeding (11, 15). We tested whether the nonviremic transmission on A2G mice resulted from SAT. Salivary gland extract (SGE) was obtained by homogenizing salivary glands isolated from partially fed, uninfected *R. appendiculatus* females collected after 6 days of feeding on uninfected guinea pigs. It was previously shown that this SGE is most active in promoting SAT of THOV in a guinea pig model (6). Salivary glands were isolated and homogenized in

TABLE 2. Relative transmission coefficients

Treatment	BALB/c		A2G	
	Tick samples[a]	Animals[b]	Tick samples	Animals
Injection of 5×10^3 PFU s.c.	24/28 (0.86)	7/7 (1.0)	0/90 (0.0)	0/20 (0.0)
Cofeeding of infected nymphs	16/23 (0.70)	7/7 (1.0)	12/44 (0.27)	5/15 (0.33)
Injection of 5×10^3 PFU + SGE s.c.	10/10 (1.0)	3/3 (1.0)	5/40 (0.13)	3/10 (0.30)

[a] Number of positive recipient tick samples/number tested (coefficient).
[b] Number of animals supporting transmission to recipient ticks/number tested (coefficient).

phosphate-buffered saline (pH 7.5) shortly before use. Virus was mixed with SGE equivalent to the salivary glands of one tick (corresponding to approximately 50 μg of total protein, as estimated by the Bio-Rad protein assay against bovine serum albumin standards) prior to s.c. injection. In this experiment, 3 in 10 A2G mice supported transmission to feeding larvae (Tables 1 and 2); the approximately twofold reduction in transmission efficiency compared with cofeeding transmission (Table 2) is consistent with previously published data (11). Again, no infection was detected in these animals, as assessed by titration of blood, liver, and spleen samples. Three similarly treated BALB/c mice developed systemic infections and supported transmission (Tables 1 and 2). Similar experiments using SGE from unfed ticks did not result in virus transmission to larvae feeding on inoculated A2G mice. These results suggest that saliva components are responsible for the cofeeding transmission observed in A2G mice, supporting the hypothesis that SAT is the underlying mechanism of nonviremic transmission.

In summary, the data reported here demonstrate that nonviremic transmission of THOV on A2G mice can occur efficiently and is most likely potentiated by a component(s) in tick saliva. The underlying mechanism may relate to the ability of tick SGE to inhibit type 1 interferon (IFN) production and, possibly, the action of IFN (2, 16). If this is so, it implies that cells exposed to tick saliva in the skin of A2G mice may not respond to released IFN, thus preventing the induction of Mx1 and the resulting antiviral state. THOV would then be able to establish a local skin infection, possibly including motile cells (e.g., Langerhans cells) that may act as part of a shuttling system, transporting virus from one tick feeding site to another, as postulated for tick-borne encephalitis virus (9). The biological significance of nonviremic transmission via *Mx1*$^+$ mice is unknown; however, *Mx1*-based resistance is found in approximately three-quarters of wild mice (3), and it is tempting to speculate that *Mx1*$^+$ alleles protect these animals from sporadic, hitherto undetected tick-borne orthomyxoviruses like THOV, as suggested previously (4). Thus, nonviremic transmission may play a key role in promoting the survival of these viruses in the natural ecosystem, as shown for tick-borne encephalitis virus (11).

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